Companion cells, a diamond in the rough

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Highlight: Companion cells are known for their role in phloem loading but they are also involved in relevant functions for plant fitness, like flowering, the clock or organ morphogenesis.

Abstract

Vascular plants have developed highly specialized cells to transport nutrients and developmental signals. The differentiation process includes the degradation of multiple organelles of the sieve element cells (SEs) to facilitate transport and as a consequence, SEs become dependent on neighboring companion cells (CCs). Despite its importance for phloem function and flowering time control, CCs are still a mysterious cell type. In this review, we gather all the genes known to be expressed in CCs, in different organs and organisms, with the objective of better understanding CC identity and function.

Key words: companion cells, development, flowering, FT, phloem, sieve element, transcriptome.

Introduction

Phloem tissue connects the aerial photosynthetic organs to heterotrophic structures like roots and fruits, distributing nutrients for adequate plant growth and fitness. In addition to transporting photoassimilates, it also influences plant development, since it distributes plant hormones, mRNA, small RNAs and proteins. To perform these fundamental functions, the sieve element cells (SEs) become a pipe following a unique differentiation process. On the one hand, the plasmodesmata (PD) in the cell walls connecting the different SEs are enlarged and the desmotubule (the structure derived from the endoplasmic reticulum in the axis of the pore), gets disintegrated, resulting in the mature sieve plate pores that will facilitate transport (Dettmer *et al.*, 2014). In

addition, SE enucleate and lose many organelles including the rough endoplasmic reticulum, vacuole, Golgi and cytoskeleton. However, SEs are still alive, keeping mitochondria, smooth endoplasmic reticulum and plastids. Due to this special condition, SEs are physiologically supported by neighboring companion cells (CCs) (Furuta *et al.*, 2014a).

The term "companion cells" (CC) was first established by Wilhelm in 1880 (from the German *geleitzelle*)(Wilhelm, 1880) to describe the nucleate cells associated with the SEs in angiosperms. CCs have dense cytoplasm, large well differentiated nuclei, absence of starch (except in senile phloem), usually minor vacuolation, high phosphatase activity and are connected to adjacent SE by multiple branched PD that allow the supply of proteins and transcripts to the SE (Cronshaw, 1981; Esau, 1969; Lucas *et al.*, 2013). Despite this general description, CCs have been historically classified in relation to the loading mechanism they use to capture nutrients:

1) **Ordinary CCs** use the apoplastic mechanism, where the sugars produced in mesophyll cells are exported to the cell wall space and then subsequently imported by transporters using energy generated by proton pumps. They look unspecialized.
2) Ordinary CCs are called **transfer cells** when they show cell ingrowths to better capture sugars.
3) CCs using polymer trapping (sugars entering CCs are converted to larger molecules of raffinose and stachyose so that they cannot escape) are known as **intermediary cells**. They are larger than ordinary CCs.
4) CCs involved in passive loading by sugar diffusion don't receive any particular name but have many symmetrically branched PD (Heo *et al.*, 2014; Slewinski *et al.*, 2013).

It was long thought that SEs and CCs originate from a common precursor cell. However, using consecutive cross sections demonstrates that, at least in the Arabidopsis root, they come from different procambium cells (Mahonen *et al.*, 2000). Next to the quiescent center (QC) the vascular initials undergo one or more anticlinal divisions. Then, cells orthogonal to the central xylem axis divide periclinally to form another procambium cell and the sieve element precursor cell. In the next periclinal

division, the precursor cell generates protophloem and metaphloem cells. On the contrary, in the case of CCs, procambium cells adjacent to the protophloem SE (two per pole), undergo one or several anticlinal divisions and then divide periclinally to produce a procambium cell and a CC (Mahonen *et al.*, 2000) (Fig. 1).

While *OCTOPUS* (*OPS*), *BREVIS RADIX* (*BRX*), *CLAVATA 3 EMBRYO SURROUNDING REGION 45* (*CLE45*) and *BARELY ANY MERISTEM 3* (*BAM3*), important genes for SE identity and differentiation (De Rybel *et al.*, 2016; Rodriguez-Villalon, 2015), are known to be expressed in phloem initials, the genes expressed in early CCs, potentially involved in CC specification, are for the moment unknown. Despite so many questions regarding the establishment and maintenance of CC identity, many proteins have shown to be exclusively or predominantly expressed in CCs, demonstrating these cells have a unique transcriptome (Tsuwamoto and Harada, 2011) (See table 1 for a short description of the proteins described below).

Proteins expressed in CCs

Proteins expressed in CCs and other locations

For the moment, ALTERED PHLOEM DEVELOPMENT (APL) is the marker known to appear earliest during CC development. It is expressed first in protophloem SEs after the asymmetric divisions and in phloem pole pericycle (PPP), the pericycle cells in contact with phloem poles. However, after enucleation it is detected only in CCs and metaphloem (Bonke *et al.*, 2003; Furuta *et al.*, 2014b). In fact, APL is required for phloem differentiation: in *apl* mutants neither SE nor CCs are found in the phloem poles. Furthermore, phloem markers like the protophloem J0701 or the CC- specific marker SUC2 are not detected in the mutant background. Even so, the first two phloem periclinal divisions, although delayed, still take place in *apl*, so APL is not required for early stages of phloem development (Bonke *et al.*, 2003).

Another protein expressed in CCs and SEs important for correct phloem development is LATERAL ROOT DEVELOPMENT 3 (LRD3), a protein containing a LIM domain about 150 amino acids from the N-terminal and a zinc binding domain close to the C-terminus. In this case, the mutant produces more lateral roots, has a smaller amount of callose (a β-1,3-glucan polymer that influences PD aperture) in the root tip, narrower sieve elements with increasing space between sieve plates, and root tip

unloading problems, defects that are corrected in older plants or when treating with auxin (Ingram *et al.*, 2011). Therefore, even if LRD3 function is unknown, LRD3 is important for early phloem development and could contribute to a correct partition of sucrose between the primary root and lateral organs (Ingram *et al.*, 2011).

A protein of unknown function, PHLOEM PROTEIN 2 (PP2), a dimeric lectin, was detected in mature CCs of pumpkin hypocotyls (Bostwick *et al.*, 1992). In *Cucurbita maxima*, vPP2 is found in both SEs and CCs (Golecki *et al.*, 1999). In turn, PP1, which forms the controversial P-protein filaments observed in electron micrographs (Behnke, 1990; Oparka and Cruz, 2000), is expressed in CCs but the protein is found mostly in SEs and only occasionally in CCs (Clark *et al.*, 1997a).

In turn, SCARECROW-LIKE 15 (SCL15), a GRAS nuclear factor in charge of repressing seed maturation program in vegetative tissue, is expressed predominantly in CCs but the specificity varies along organs and time of development. While it is expressed in CCs of chalazal ends of ovule foniculi and also in CCs and bundle sheath of leaf petioles, in the root tip it is however expressed in SEs and PPPs. Interestingly, in mature roots the expression is maintained in PPPs but is found first in SEs and then in CCs (Gao *et al.*, 2015), a situation reminiscent of the APL expression pattern. Indeed, based on these expression patterns, it is tempting to speculate that the SE could be acting as a source of mobile signals before enucleation, a role that would be taken over by CCs when SE differentiates.

Proteins mostly specific to CCs

The genes identified to be specific of CCs are mostly found in mature cells and are related to transport, which is an essential function for CCs that are in charge of loading and unloading substances into the SE.

Arabidopsis H+-ATPase isoform 3 (AHA3) is a membrane proton pump specific to CCs in leaf and stem transections of Arabidopsis (DeWitt *et al.*, 1991; DeWitt and Sussman, 1995). In addition to maintaining the osmotic and chemical balance in the cytoplasm by coupling ATP hydrolysis to ion transportation, it is thought to provide the energy necessary for sugar uptake.

On the contrary, proton-pumping pyrophosphatase (H+ -PPases) usually use the proton motive force to produce more PPi and promote sucrose oxidation and ATP synthesis in CCs. Type 1 ARABIDOPSIS VACUOLAR PYROPHOSPHATASE1 (AVP1) is

found in xylem vessels and in the plasma membrane of SE and CC in source leaves of Arabidopsis (Paez-Valencia *et al.*, 2011). However, overexpressing this protein under a constitutive or a CC-specific promoter had the same effects (increased biomass accumulation, phloem loading and long-distant transport), pointing to a prominent role in CCs (Khadilkar *et al.*, 2016).

A few years after AHA3 was discovered, it was shown that the *SUCROSE PROTON SYMPORTER 2* gene, *SUC2*, essential for Arabidopsis development (Gottwald *et al.*, 2000) was found exclusively expressed in the plasma membrane of CCs, mimicking *AHA3* expression pattern (Truernit and Sauer, 1995). It was present in source leaves and the mature root, but not in the root tip, sink leaves, petals or ovules, linking this gene to phloem loading (Truernit and Sauer, 1995). Due to its specificity and strong expression it has been a valuable tool to study CCs and phloem loading/unloading. For instance, free movement of small proteins from CCs to SEs was proved by expressing GFP under the control of this promoter, finding GFP unloaded into the sink tissues where *SUC2* is not expressed (Imlau *et al.*, 1999). This also helped define the size exclusion limit of PD, that decreases along organ development (Imlau *et al.*, 1999; Oparka and Turgeon, 1999), further demonstrated when used to express fluorescent proteins of different sizes (Stadler *et al.*, 2005).

Other sucrose transporters, like *SUCROSE TRANSPORTER 1*, SUT1, and *SUCROSE TRANSPROTER 4*, SUT4, are expressed in CCs but the proteins localize to the SEs, a situation found in different species of dicots (Kuhn *et al.*, 1997; Weise *et al.*, 2000). The current model suggests the movement of both mRNA and SUT1 protein through the desmotubule connecting CCs and SEs (Liesche *et al.*, 2011), although movement to the SEs is controversial (Schmitt *et al.*, 2008). In dicots SUT1 is involved in phloem loading (Kuhn C. Quick WP, 1996; Lemoine R, 1996). However, in monocots the importance of SUT1 remains unclear: in sugarcane (*Saccharum hybrid*) *SUT1* is not expressed in the phloem (Rae *et al.*, 2005) and in rice (*Oryza sativa*), *SUT1* is expressed in different phloem cell types (Scofield *et al.*, 2007) but it is not essential for carbon partitioning (Ishimaru K, 2001; Scofield GN, 2002) and is suggested to be involved in sucrose recovery after loss to the apoplasm during transport or after aphid attack (Ibraheem *et al.*, 2014; Scofield *et al.*, 2007) . However, the role of *SUT1* for phloem loading in maize has been clearly demonstrated in two different alleles of *sut1* mutants (Slewinski *et al.*,

2010; Slewinski *et al.*, 2009). Both alleles have decreased growth, hyperaccumulate carbohydrates within mature leaves, and decreased 14C-labeled sucrose export. Interestingly, *sut1-m4* secretes a concentrated sugar solution from leaf hydathodes (Slewinski *et al.*, 2010). These specialized leaf structures usually secrete only water in a process caused by root pressure (guttation). Exceptionally, they secrete sugars when phloem transport is prevented after cold-girdling, a treatment consisting on cooling a part of the plant by putting it in contact with a tube transporting cold water (Slewinski *et al.*, 2009). Recently, SUT1 protein in maize has been shown to localize in the plasma membrane of CC but also in all other cell types included within the vascular bundle, except SE and xylem vessels, overlapping with the expression domain (promoter fusion) and *in situ* data (Baker *et al.*, 2016). On top of this, *ZmSUT1* has been also found expressed in all sink organs without correlation with the phloem unloading zone (Baker *et al.*, 2016). These data and the fact that it is localized in non-conducting cells points to an additional role of SUT1 in sucrose retrieval as previously suggested for rice (Baker *et al.*, 2016; Ibraheem *et al.*, 2014; Scofield *et al.*, 2007).

Sucrose is not the only molecule CCs take on. *SULTR1,3* sulfate transporter is expressed exclusively in mature CCs of the root. Its expression spreads to CC-SE in mature organs like cotyledons and interestingly it was not found in sink organs like young rosette leaves. Contrary to other sulfate transporters discovered in Arabidopsis, SULTR1,3 is not in charge of the initial sulfate uptake by the root epidermis and cortex, but in mediating sulfate phloem transport, preventing any leakage from the SE and maintaining sulfur flux (Yoshimoto *et al.*, 2003). Another sulphate transporter, SULTR2-1, is also mostly specific of CC (Zhang *et al.*, 2008).

In turn, SODIUM POTASSIUM ROOT DEFECTIVE1 (NaKR1), also called NPCC6, is a soluble cytoplasmic protein with a heavy metal-associated binding domain (HMA) expressed specifically in mature CCs but able to move to other cell types, even if movement doesn´t seem to be essential for its function (Tian *et al.*, 2010). Interestingly, *nakr1* mutant shows pleiotropic defects: even if phloem is normally differentiated, *nakr1* has problems in phloem loading or translocation, an excess of Na^+, K^+, Rb^+ and starch in the leaves, shorter meristem and it is late flowering. Recently, NaKR1 function has been partially clarified: it is activated by CONSTANS (CO) and binds to FLOWERING LOCUS T (FT) through its HMA domain for long-distance

transport to the shoot apex under long-day conditions (Zhu *et al.*, 2016). As the authors speculate, NaKR1 could be also involved in coordinating sucrose transport to ensure a successful flowering, an energy consuming event for the plant.

Lastly, an enzyme involved in the first step of raffinose and stachyose synthesis, the galactinol synthase promoter (*GAS*) of melon (*Cucumis melo*) is localized mostly in CCs of mature leaves in Arabidopsis and tobacco. In tobacco class-V minor veins, three CCs surround 2 SEs. Interestingly, *GAS* expression is only detected in one SE and two of the CCs surrounding it, indicating different CC transcriptomes within CCs in the same vein and among different veins (Haritatos *et al.*, 2000). On the contrary *SUC2* is expressed in both minor and major veins.

CC proteins and viruses

CCs are also the entrance for some viruses to the SE pipe that will take them to invade other healthy tissues. AUXIN INDOLE ACETIC ACID 26 (Aux/IAA26) is found in the nuclei of CCs in older leaves of *A. thaliana* accession *Shahdara* and it is known to bind the replication protein of tobacco mosaic virus (TMV). In addition, it is one of the IAA proteins that interacts with auxin-responsive transcription factors (ARFs) and when degraded by auxin, triggers auxin responsive genes. Interestingly, when infected with TMV, the nuclear localization of Aux/IAA26 is disrupted, resulting in enhanced phloem loading and viral systemic movement suggesting the possibility that the virus is reprogramming the vascular transcriptome to promote infection (Collum *et al.*, 2016).

Another ssRNA virus, *cucumber mosaic virus* (CMV) interacts with cucumber protein p48, a homolog of pumpkin PP1, becoming more resistant to RNase (Requena *et al.*, 2006). This way, the interaction with p48 would change the structure of CMV particles providing some advantage for the infection.

However, cadmium-ion-induced GRP (cdiGRP), a tobacco glycine-rich protein expressed in the cell wall of SEs and CC, is induced by low concentrations of cadmium and might be involved in the control of *turnip vein-clearing tobamovirus* (TVCV) systemic movement: when cdiGRP is overexpressed, TVCV cannot leave the vascular bundles and an increase in callose deposition in the vasculature is observed (Hipper *et al.*, 2013; Ueki and Citovsky, 2002).

On the other hand, the *commelina yellow mottle virus* (CoYMV), a badnavirus that infects the monocot *Commelina diffusa*, has been used as a tool to study CCs, since its

promoter is expressed in CCs in many organs of tobacco (Khadilkar *et al.*, 2016; Matsuda *et al.*, 2002; Medberry *et al.*, 1992; Tsuwamoto and Harada, 2011).

Large-scale attempts to profile the CC-transcriptome

The small size and the difficult position of CCs, embedded within the vascular cylinder, have hampered CC transcriptomics.

The first attempt used SUC2 protoplasts from vascular strands of rosette leaves. GFP positive protoplasts were selected manually and an EST library was constructed. Transcripts from the described markers SUC2 and AHA3 were used as a positive control to validate the samples and around 700 ESTs from CCs were published and categorized in different clusters, mostly highlighting the active metabolic functions developed by CCs, as a nursing cell for SEs. However, the specificity of these transcripts was not validated (Ivashikina *et al.*, 2003).

In a second study, this time in the Arabidopsis root, SUC2 was chosen as the marker for mature CCs, described as expressed from elongating CCs all along the root till the hypocotyl, GFP becoming stronger once the root hair is obvious. Root protoplasts were sorted and the RNA was hybridized in microarrays. There are more than 7000 entries in the top 50% varying genes and transcripts like AHA3 and SUC2 are found in this list (Brady *et al.*, 2007). The transcripts included are enriched in CCs but might not be specific of this cell type. Cells expressing APL were also profiled in this study.

Using a similar approach, root protoplasts expressing SULTR2-1, a sulfate transporter mostly expressed in CCs, were sorted and 12 enriched genes were selected for further analysis. Even if not all of them were CC specific when validated through transcriptional reporter fusions, the list included NaKR1, pointed to be CC specific in this study for the first time (Zhang *et al.*, 2008).

Using a different less specific approach, Tsuwamoto and Harada used vascular strings with a high number of CCs from petioles of *Brassica napus*, a close relative of Arabidopsis. With this material they constructed a subtracted cDNA library to find ESTs enriched in CCs. To validate their approach they focus on the CORI3 Arabidopsis promoter, the Arabidopsis homolog of the most frequent EST in the library, and found it is expressed in vascular tissue including CCs. The corresponding Arabidopsis homologs to the ESTs found are also listed in this publication (Tsuwamoto and Harada, 2011).

Not only nurses

The role of CCs in nutrient loading and unloading has been extensively reviewed (Ham and Lucas, 2014; Lucas *et al.*, 2013; Oparka and Cruz, 2000; Slewinski *et al.*, 2013; van Bel *et al.*, 2002). However, CCs do not only nurse and deliver but they are the key cell type for a function essential for plant success: determining the appropriate time for flowering (Fig. 2).

Indeed, the long-sought florigen (Chailakhyan, 1936; Sachs, 1865), FT, is expressed in CCs of the leaves and cotyledons under long day conditions and then travels to the shoot apical meristem (SAM) to activate together with FLOWERING LOCUS D (FD) several floral meristem identity factors and induce flowering (Corbesier *et al.*, 2007; Jaeger and Wigge, 2007; Mathieu *et al.*, 2007; Wigge *et al.*, 2005).

FT expression is activated by CONSTANS (CO) (An *et al.*, 2004). In turn, *CO* is repressed by several cycling DOFs (CYCLING DOF FACTOR, CDF1-3 and CDF5): when misexpressed in CCs, plants flower later and on the contrary the quadruple mutant is insensitive to photoperiod and flowers early due to a high expression of *CO* (Fornara *et al.*, 2009).

As mentioned above, *CO* not only activates *FT* but *NaKR1*, in charge of long-distance transport of FT (Ahn, 2016; Zhu *et al.*, 2016) to the SAM. Before this step, FT interacts with FT INTERACTING PROTEIN 1 (FTIP1) in CCs and is then exported to the SE (Liu *et al.*, 2012).

Recently, it was also discovered that a classic late-flowering mutant, *fe*, is indeed another allele of *apl*. APL would simultaneously activate *FT* and *FTIP1* expression, activating not only the transcription of the florigen but also at least part of the transport machinery (Abe *et al.*, 2015).

Another way to stimulate FT expression is by downregulating *FLOWERING LOCUS C* (*FLC*), a MADS-box transcription factor that integrates several flowering pathways and a repressor of FT (Cao *et al.*, 2008; Crevillen *et al.*, 2014). Interestingly, a JmjC-domain containing histone H3K4 demethylase, JMJ18, expressed in the stele including CCs,

reduces H3K4 methylation in FLC chromatin, leading to *FLC* repression, a circumstance that will promote *FT* transcription and flowering (Yang *et al.*, 2012).

Adding another layer of complexity, FT exhibits circadian expression, showing a peak at dusk. However, a reduced expression of FT in the evening is observed when CIRCADIAN CLOCK ASSOCIATED 1 (CCA1), one of the clock genes, is expressed in CCs under the SUC2 promoter. Consistently, these plants are late flowering. However, when CCA1 is expressed under a xylem or (pro)cambium promoter plants flower normally, demonstrating that the clock sensing photoperiodic cues is based in CCs, attributing a new function for this cell type. Contrary to animals, the clock in Arabidopsis is not centralized but is tissue-specific, with the epidermal clock in charge of sensing darkness and promote hypocotyl cell elongation (Shimizu *et al.*, 2015).

In addition to flowering induction, FT promotes tuber formation in potato (*Solanum tuberosum*), with two *FT*-like paralogues from the *SELF-PRUNING* gene family controlling floral and tuberization transitions (Navarro *et al.*, 2011). Therefore, the non-cell autonomous function of the mobile FT protein is not restricted to flowering, involving the CCs in another key process in potato, the third largest food crop in the world.

Another gene expressed in CCs with a remarkable function is *CLAVATA1* (*CLV1*), that codes for a leucine-rich repeat receptor-like kinase known for regulating meristematic activity and stem cell differentiation in the SAM (Clark *et al.*, 1997b; Nimchuk *et al.*, 2011; Schoof *et al.*, 2000). Upon nitrogen deficient conditions, CLAVATA3/ESR-related (CLE) peptides, expressed in pericycle cells, and CLV1 in the membrane of CCs, constitute the signalling module repressing the outgrowth of lateral root primordia and their emergence, an important feature to avoid root extension to nitrogen poor soils (Araya *et al.*, 2014). This example highlights the relevance of the communication between CCs and other neighbouring cell types, different from SEs, and implicate CCs in root development.

Altogether, the cases listed involve CCs in meristem-associated transitions (flowering, tuber formation), the clock sensing photoperiod and the translation of an environmental cue into a root morphological response, functions crucial for plant fitness and success.

Future perspectives

Neurons have been traditionally considered the only relevant cells in the brains of mammals. On the contrary, astrocytes were thought to be simple passive cells with a mere neuron supportive function. Only in recent years the complexity and functional diversity of these cells is being uncovered, with roles in ion homeostasis, neurotransmitter clearance, and synapse formation and removal (Allen and Barres, 2009; Khakh and Sofroniew, 2015).

However, in plants, CC function is still obscure. Little is known about CC specification, how the CC transcriptome and proteome changes along development or the functions carried out in different organs. In addition, the intimate relationship between CCs and SEs remains almost unexplored, a question worth answering since probably CC cannot be understood without SE and *vice versa*, although it is still too early to know if SE-CCs form a functional unit.

Understanding CC identity and function is an essential piece to complete the vascular puzzle, a tissue essential for plant fitness in charge of inter-organ connection and communication in plants.

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Protein name [1]	Gene ID in Arabidopsis	Function	Found/expressed in	References (a selection)[2]
APL	AT1G79430	Phloem differentiation. Promotes FT expression	Protophloem SE, metaphloem SE and CC	Bonke et al. 2003 Furuta et al. 2014
AHA3	AT5G57350	Ion homeostasis/energy for sugar intake	CC	DeWitt *et al.*, 1991; DeWitt and Sussman, 1995
Aux/IAA26	AT3G16500	Allow auxin responsive genes to activate	CC nuclei of old leaves	Collum *et al.*, 2016
AVP1	AT1G15690	Produce more PPi and promote sucrose oxidation and ATP synthesis	Xylem vessels, SE and CC plasma membrane	Paez-Valencia *et al.*, 2011 Khadilkar *et al.*, 2016
cdiGRP		Control of virus systemic movement	SE and CC	Hipper *et al.*, 2013; Ueki and Citovsky, 2002
CLV1	AT1G75820	Controls lateral root outgrowth and emergence under nitrogen deficient conditions together with CLE peptides	CC	Araya *et al.*, 2014
CO	AT5G15840	Activates FT expression	CC	An *et al.*, 2004 Fornara *et al.*, 2009
CORI3	AT4G23600	Hormone and stress response	Vasculature	Tsuwamoto and Harada, 2011
FT	AT1G65480	Florigen. Induces tuberization in potato. Photoperiod clock.	CC. It travels through SE from leaves to SAM	Corbesier *et al.*, 2007 Navarro *et al.*, 2011
GAS		Galactinol synthase	Some CC of the minor veins of mature leaves	Haritatos *et al.*, 2000
JMJ18	AT1G30810	Histone H3K4 demethylase	Stele including CC	Yang *et al.*, 2012
LRD3	AT2G39830	Unknown	CC and SE	Ingram et al. 2011
NaKR1		Long distance	Mature CCs. It	Tian *et al.*,

	AT5G02600	transport of FT and other unknown functions	moves.	2010 Zhu *et al.*, 2016
PP1		Unknown	SE and occasionally CC	Clark *et al.*, 1997
PP2		Unknown	Mature CC and SE	Bostwick *et al.*, 1992 Golecki *et al.*, 1999
SCL15	AT4G36710	Repress seed maturation program	CC and other layers	Gao *et al.*, 2015
SUC2	AT1G22710	Sucrose transporter. Phloem loading	CC	Truernit and Sauer, 1995
SULTR1,3	AT1G22150	Sulfate transporter. Prevent sulfur leakage from SE	Mature CC of the root	Yoshimoto *et al.*, 2003
SUT1		Sucrose transporter. Phloem loading in dicots and maize	Expressed in CC. Probably moves to SE	Kuhn *et al.*, 1997 Slewinski *et al.*, 2010
SUT4	AT1G09960	Sucrose transporter	Expressed in CC. Probably moves to SE.	Weise *et al.*, 2000

Table 1. Proteins expressed in CCs.

[1] Full name is provided in the text.

[2] References are included in the reference list.

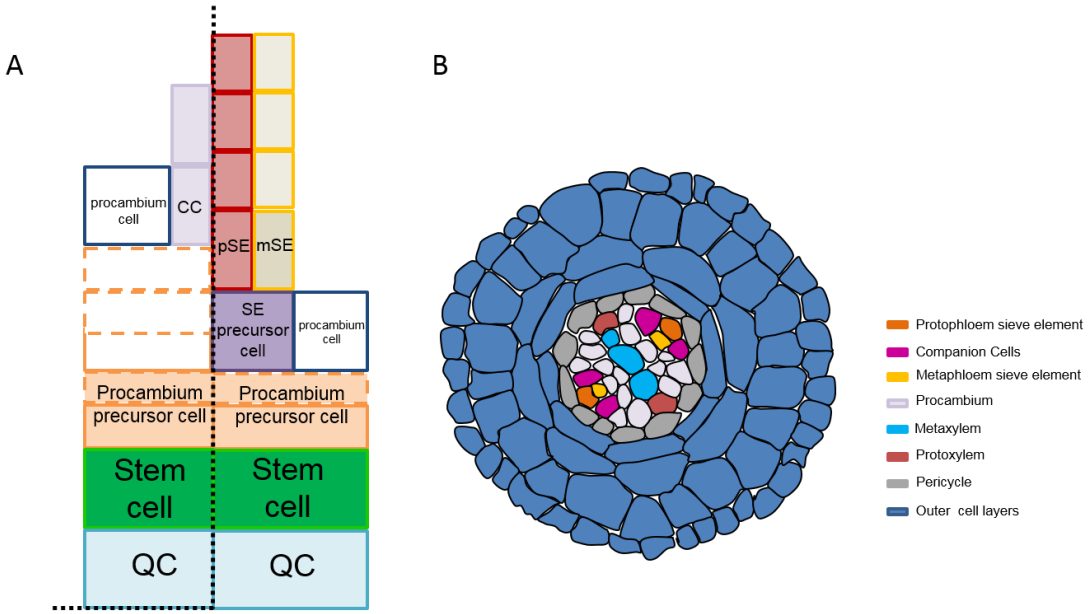

Fig 1. Vascular morphogenesis in the Arabidopsis root. A) The vascular initials in contact with the QC (stem cells) divide anticlinally once or several times. Two periclinal divisions generate the SEs: first the procambium precursor cell divides periclinally to generate and SE precursor cell and a procambium cell and then the SE precursor cell undergoes another periclinal division to generate protophloem SE (pSE) and metaphloem SE (mSE). On the contrary, in the case of CCs, procambium cells adjacent to the protophloem SE (orthogonal position indicated by dashed line), undergo one or several anticlinal divisions and then divide periclinally to produce a procambium cell and a CC (Mahonen *et al.*, 2000). B)) A cross-section of the root to facilitate the comprehension of the spatial distribution of the cells.

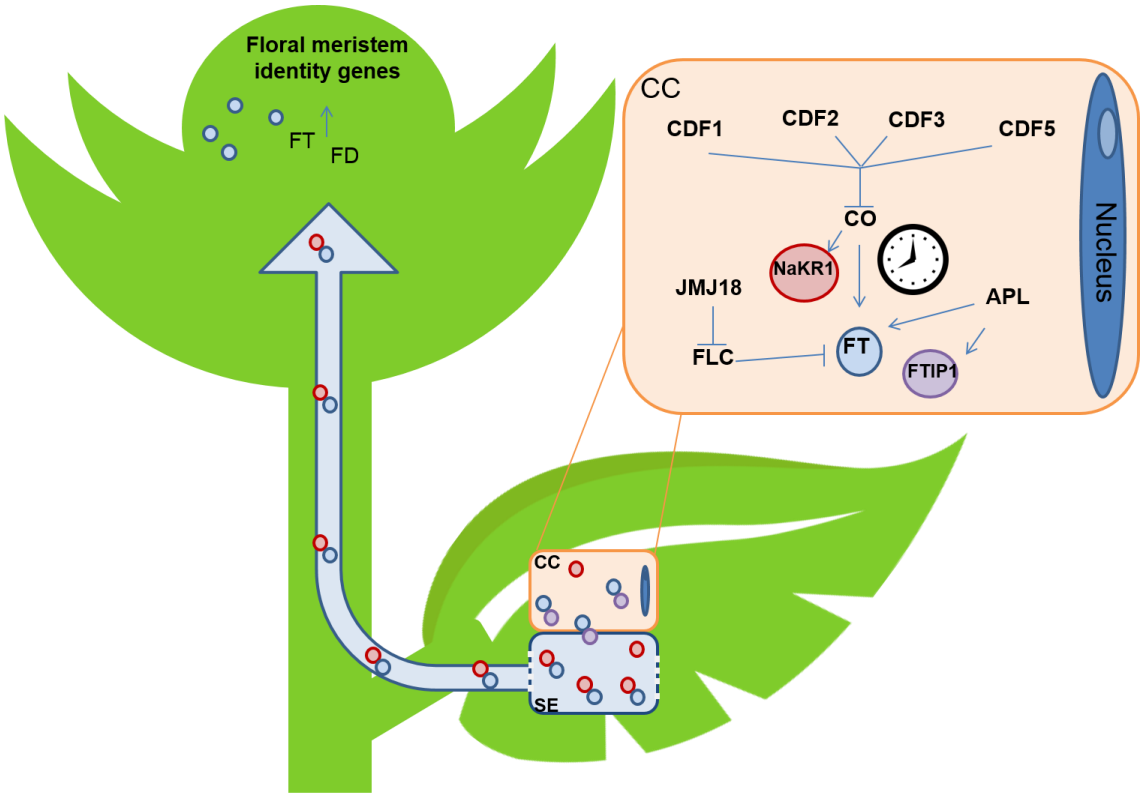

Fig 2. CCs are a key cell type for photoperiodic flowering regulation under long day conditions. CO, that is repressed by several cycling DOFs (CDF1-3; CDF5), activates the expression of both FT (blue circle), the florigen, and NaKR1 (red circle) in CC. In turn, APL promotes the expression of FT and also FTIP1 (purple circle), in charge of exporting FT to the SEs. NaKR1 will take FT to the SAM and there FT and FD will activate the expression of floral meristem identity genes. FT expression is also promoted by a downregulation of FLC expression through histone H3K4 demethylase JMJ18. In addition, the circadian clock regulates the time of flowering through FT-CO.